

GSK-3 $\beta$  Sequence

Met	Ser	Gly	Arg	Pro	Arg	Thr	Thr	Ser	Phe	Ala	Glu	Ser	Cys	Lys	Pro
1				5					10					15	
Val	Gln	Gln	Pro	Ser	Ala	Phe	Gly	Ser	Met	Lys	Val	Ser	Arg	Asp	Lys
	20						25					30			
Asp	Gly	Ser	Lys	Val	Thr	Thr	Val	Val	Ala	Thr	Pro	Gly	Gln	Gly	Pro
	35						40					45			
Asp	Arg	Pro	Gln	Glu	Val	Ser	Tyr	Thr	Asp	Thr	Lys	Val	Ile	Gly	Asn
	50						55					60			
Gly	Ser	Phe	Gly	Val	Val	Tyr	Gln	Ala	Lys	Leu	Cys	Asp	Ser	Gly	Glu
65				70					75					80	
Leu	Val	Ala	Ile	Lys	Lys	Val	Leu	Gln	Asp	Lys	Arg	Phe	Lys	Asn	Arg
	85						90					95			
Glu	Leu	Gln	Ile	Met	Arg	Lys	Leu	Asp	His	Cys	Asn	Ile	Val	Arg	Leu
	100						105					110			
Arg	Tyr	Phe	Phe	Tyr	Ser	Ser	Gly	Glu	Lys	Lys	Asp	Glu	Val	Tyr	Leu
	115						120					125			
Asn	Leu	Val	Leu	Asp	Tyr	Val	Pro	Glu	Thr	Val	Tyr	Arg	Val	Ala	Arg
	130						135					140			
His	Tyr	Ser	Arg	Ala	Lys	Gln	Thr	Leu	Pro	Val	Ile	Tyr	Val	Lys	Leu
145				150					155					160	
Tyr	Met	Tyr	Gln	Leu	Phe	Arg	Ser	Leu	Ala	Tyr	Ile	His	Ser	Phe	Gly
	165						170					175			
Ile	Cys	His	Arg	Asp	Ile	Lys	Pro	Gln	Asn	Leu	Leu	Leu	Asp	Pro	Asp
	180						185					190			
Thr	Ala	Val	Leu	Lys	Leu	Cys	Asp	Phe	Gly	Ser	Ala	Lys	Gln	Leu	Val
	195						200					205			

*Fig. 1A*

Arg Gly Glu Pro Asn Val Ser Tyr Ile Cys Ser Arg Tyr Tyr Arg Ala  
210 215 220

Pro Glu Leu Ile Phe Gly Ala Thr Asp Tyr Thr Ser Ser Ile Asp Val  
225 230 235 240

Trp Ser Ala Gly Cys Val Leu Ala Glu Leu Leu Leu Gly Gln Pro Ile  
245 250 255

Phe Pro Gly Asp Ser Gly Val Asp Gln Leu Val Glu Ile Ile Lys Val  
260 265 270

Leu Gly Thr Pro Thr Arg Glu Gln Ile Arg Glu Met Asn Pro Asn Tyr  
275 280 285

Thr Glu Phe Lys Phe Pro Gln Ile Lys Ala His Pro Trp Thr Lys Val  
290 295 300

Phe Arg Pro Arg Thr Pro Pro Glu Ala Ile Ala Leu Cys Ser Arg Leu  
305 310 315 320

Leu Glu Tyr Thr Pro Thr Ala Arg Leu Thr Pro Leu Glu Ala Cys Ala  
325 330 335

His Ser Phe Phe Asp Glu Leu Arg Asp Pro Asn Val Lys His Pro Asn  
340 345 350

Gly Arg Asp Thr Pro Ala Leu Phe Asn Phe Thr Thr Gln Glu Leu Ser  
355 360 365

Ser Asn Pro Pro Leu Ala Thr Ile Leu Ile Pro Pro His Ala Arg Ile  
370 375 380

Gln Ala Ala Ala Ser Thr Pro Thr Asn Ala Thr Ala Ala Ser Asp Ala  
385 390 395 400

Asn Thr Gly Asp Arg Gly Gln Thr Asn Asn Ala Ala Ser Ala Ser Ala  
405 410 415

Ser Asn Ser Thr  
420

*Fig. 1B*

**GSK-3 $\beta$  557 Construct Sequence**  
**Met Glu Tyr Met Pro Met Glu Gly Gly Gly**

Met Ser Gly Arg Pro Arg Thr Thr Ser Phe Ala Glu Ser Cys Lys Pro  
1 5 10 15

Val Gln Gln Pro Ser Ala Phe Gly Ser Met Lys Val Ser Arg Asp Lys  
20 25 30

Asp Gly Ser Lys Val Thr Thr Val Val Ala Thr Pro Gly Gln Gly Pro  
35 40 45

Asp Arg Pro Gln Glu Val Ser Tyr Thr Asp Thr Lys Val Ile Gly Asn  
50 55 60

Gly Ser Phe Gly Val Val Tyr Gln Ala Lys Leu Cys Asp Ser Gly Glu  
65 70 75 80

Leu Val Ala Ile Lys Lys Val Leu Gln Asp Lys Arg Phe Lys Asn Arg  
85 90 95

Glu Leu Gln Ile Met Arg Lys Leu Asp His Cys Asn Ile Val Arg Leu  
100 105 110

Arg Tyr Phe Phe Tyr Ser Ser Gly Glu Lys Lys Asp Glu Val Tyr Leu  
115 120 125

Asn Leu Val Leu Asp Tyr Val Pro Glu Thr Val Tyr Arg Val Ala Arg  
130 135 140

His Tyr Ser Arg Ala Lys Gln Thr Leu Pro Val Ile Tyr Val Lys Leu  
145 150 155 160

Tyr Met Tyr Gln Leu Phe Arg Ser Leu Ala Tyr Ile His Ser Phe Gly  
165 170 175

Ile Cys His Arg Asp Ile Lys Pro Gln Asn Leu Leu Leu Asp Pro Asp  
180 185 190

Thr Ala Val Leu Lys Leu Cys Asp Phe Gly Ser Ala Lys Gln Leu Val  
195 200 205

Arg Gly Glu Pro Asn Val Ser Tyr Ile Cys Ser Arg Tyr Tyr Arg Ala  
210 215 220

***Fig. 2A***

Pro Glu Leu Ile Phe Gly Ala Thr Asp Tyr Thr Ser Ser Ile Asp Val  
225                      230                      235                      240

Trp Ser Ala Gly Cys Val Leu Ala Glu Leu Leu Gly Gln Pro Ile  
                    245                      250                      255

Phe Pro Gly Asp Ser Gly Val Asp Gln Leu Val Glu Ile Ile Lys Val  
                    260                      265                      270

Leu Gly Thr Pro Thr Arg Glu Gln Ile Arg Glu Met Asn Pro Asn Tyr  
                    275                      280                      285

Thr Glu Phe Lys Phe Pro Gln Ile Lys Ala His Pro Trp Thr Lys Val  
                    290                      295                      300

Phe Arg Pro Arg Thr Pro Pro Glu Ala Ile Ala Leu Cys Ser Arg Leu  
305                      310                      315                      320

Leu Glu Tyr Thr Pro Thr Ala Arg Leu Thr Pro Leu Glu Ala Cys Ala  
                    325                      330                      335

His Ser Phe Phe Asp Glu Leu Arg Asp Pro Asn Val Lys His Pro Asn  
                    340                      345                      350

Gly Arg Asp Thr Pro Ala Leu Phe Asn Phe Thr Thr Gln Glu Leu Ser  
                    355                      360                      365

Ser Asn Pro Pro Leu Ala Thr Ile Leu Ile Pro Pro His Ala Arg Ile  
                    370                      375                      380

*Fig. 2B*

**GSK-3 $\beta$  580 Construct Sequence**  
**Met Glu Tyr Met Pro Met Glu Gly Gly Gly**

Gly	Ser	Lys	Val	Thr	Thr	Val	Val	Ala	Thr	Pro	Gly	Gln	Gly	Pro
	35						40					45		
Asp	Arg	Pro	Gln	Glu	Val	Ser	Tyr	Thr	Asp	Thr	Lys	Val	Ile	Gly
	50						55					60		Asn
Gly	Ser	Phe	Gly	Val	Val	Tyr	Gln	Ala	Lys	Leu	Cys	Asp	Ser	Gly
65				70					75					80
Leu	Val	Ala	Ile	Lys	Lys	Val	Leu	Gln	Asp	Lys	Arg	Phe	Lys	Asn
	85						90					95		Arg
Glu	Leu	Gln	Ile	Met	Arg	Lys	Leu	Asp	His	Cys	Asn	Ile	Val	Arg
	100						105					110		Leu
Arg	Tyr	Phe	Phe	Tyr	Ser	Ser	Gly	Glu	Lys	Lys	Asp	Glu	Val	Tyr
	115						120					125		Leu
Asn	Leu	Val	Leu	Asp	Tyr	Val	Pro	Glu	Thr	Val	Tyr	Arg	Val	Ala
	130						135					140		Arg
His	Tyr	Ser	Arg	Ala	Lys	Gln	Thr	Leu	Pro	Val	Ile	Tyr	Val	Lys
145					150				155					160
Tyr	Met	Tyr	Gln	Leu	Phe	Arg	Ser	Leu	Ala	Tyr	Ile	His	Ser	Phe
	165						170					175		Gly
Ile	Cys	His	Arg	Asp	Ile	Lys	Pro	Gln	Asn	Leu	Leu	Leu	Asp	Pro
	180						185					190		Asp
Thr	Ala	Val	Leu	Lys	Leu	Cys	Asp	Phe	Gly	Ser	Ala	Lys	Gln	Leu
	195						200					205		Val
Arg	Gly	Glu	Pro	Asn	Val	Ser	Tyr	Ile	Cys	Ser	Arg	Tyr	Tyr	Arg
	210						215					220		Ala
Pro	Glu	Leu	Ile	Phe	Gly	Ala	Thr	Asp	Tyr	Thr	Ser	Ser	Ile	Asp
225					230				235					240

***Fig. 3A***

Trp Ser Ala Gly Cys Val Leu Ala Glu Leu Leu Leu Gly Gln Pro Ile  
245 250 255

Phe Pro Gly Asp Ser Gly Val Asp Gln Leu Val Glu Ile Ile Lys Val  
260 265 270

Leu Gly Thr Pro Thr Arg Glu Gln Ile Arg Glu Met Asn Pro Asn Tyr  
275 280 285

Thr Glu Phe Lys Phe Pro Gln Ile Lys Ala His Pro Trp Thr Lys Val  
290 295 300

Phe Arg Pro Arg Thr Pro Pro Glu Ala Ile Ala Leu Cys Ser Arg Leu  
305 310 315 320

Leu Glu Tyr Thr Pro Thr Ala Arg Leu Thr Pro Leu Glu Ala Cys Ala  
325 330 335

His Ser Phe Phe Asp Glu Leu Arg Asp Pro Asn Val Lys His Pro Asn  
340 345 350

Gly Arg Asp Thr Pro Ala Leu Phe Asn Phe Thr Thr Gln Glu Leu Ser  
355 360 365

Ser Asn Pro Pro Leu Ala Thr Ile Leu Ile Pro Pro His Ala Arg Ile  
370 375 380

*Fig. 3B*

Human GSK3 $\alpha$

MSGGGPSGGG PGSGRARTS SFAEPGGGG GGGGGPGGSA SGPGGTGGGK  
1 50  
ASVGAMGGGV GASSSGGGPG GSGGGGSGGP GAGTSFPPPG VKLGRDSGKV  
51 100  
TTVVATLGQG PERSQEVAYT DIKVINGSF GVYQARLAE TRELVAIKKV  
101 150  
LQDKRFKNRE LQIMRKLDHC NIVRLRYFFY SSGEKKDELY LNLVLEYVPE  
151 200  
TVYRVARHFT KAKLTIPILY VKVYMYQLFR SLAYIHSQGV CHRDIKPQNL  
201 250  
LVDPDTAVLK LCDFGSAKQL VRGEPNVSYI CSRYRAPEL IFGATDYTSS  
251 300  
IDVMSAGCVL AELLGQPIF PGDSGVDQLV EIIKVLGTPT REQUIREMNP  
301 350  
YTEFKFPQIK AHPWTKVFKS RTPPEAIALC SSLEYTPSS RLSPLEACAH  
351 400  
SFFDELRLCG TQLPNNRPLP PLFNFSAGEL SIQPSLNAIL IPPHLRSPAG  
401 450  
TTTLTPSSQA LTETPTSSDW QSTDATPTLT NSS  
451 483

*Fig. 4*

Human GSK3 $\alpha$

MSGGGPSGGG PGSSGRARTS SFAEPGGGG GGGGPGGSA SGPGGTGGGK  
1 50  
ASVGAMGGGV GASSSGGGPG GSGGGSGGP GAGTSFPPPG VKLGRDSGKV  
51 100  
TTVVATLGQG PERSQEVAYT DIKVINGSF GVYQARLAE TRELVAIKV  
101 150  
LQDKRFKNRE LQIMRKLDHC NIVRLRYFFY SSGEKKDELY LNLVLEYVPE  
151 200  
TVYRVARHFT KAKLTIPILY VKVYMYQLFR SLAYIHSQGV CHRDIKPQNL  
201 250  
LVDPDTAVLK LCDFGSAKQL VRGEPNVSYI CSRYYRAPEL IFGATDYTSS  
251 300  
IDVWSAGCVL AELLGQPIF PGDSGVDQLV EIIKVLGTPT REQUIREMNP  
301 350  
YTEFKFPQIK AHPWTKVFKS RTPPEAIALC SSLLEYTPSS RLSPLEACAH  
351 400  
SFFDELRLCG TQLPNNRPLP PLFNFSAGEL SIQPSLNAIL IPPHLRS  
401

*Fig. 5*



Human GSK3 $\alpha$

	<u>SGKV</u>
TTVVATLGQG PERSQEVAYT DIKVINGSF GVVYQARLAE TRELVAIKKY	100
101	150
LQDKRFKNRE LQIMRKLDHC NIVRLRYFFY SSGEKKDELY LNLVLEYVPE	
151	200
TVYRVARHFT KAKLTIPILY VKVYMYQLFR SLAYIHSQGV CHRDIKPQNL	
201	250
LVDPDATVLK LCDFGSAKQL VRGEPNVSYI CSRYRAPEL IFGATDYTSS	
251	300
IDVWSAGCVL AELLGQPIF PGDSGVDQLV EIIKVLGTPT REQUIREMNP	
301	350
YTEFKFPQIK AHPWTKVFKS RTPPEAIALC SSLLEYTPSS RLSPLEACAH	
351	400
SFFDELRCGL TQLPNNRPLP PLFNFSAGEL SIQPSLNAIL IPPHLRSPAG	
401	450
TTTLTPSSQA LTETPTSSDW QSTDATPTLT NSS	
451	483

*Fig. 6*

Human GSK3 $\alpha$

	<u>SGKV</u>
TTVWATLGQG PERSQEVAYT DIKVINGSGF GVYQARLAE TRELVAIKKV	100
101	150
LQDKRFKNRE LQIMRKLDHC NIVRLRYFFY SSGEKKDELY LNLVLEYVPE	
151	200
TVYRVARHFT KAKLTIPILY VKVYMYQLER SLAYIHSQGV CHRDIKPQNL	
201	250
LVDPDTAVLK LCDFGSAKQL VRGEPNVSXI CSRYRAPEL IFGATDYTSS	
251	300
IDVMSAGCVL AELLGQPIF PGDSGVDQLV EIIKVLGTPT REQUIREMNPV	
301	350
YTEFKFPQIK AHPWTKVFKS RTPPEAIALC SSLLEYTPSS RLSPLEACAH	
351	400
SFFDELRLCG TQLPNNRPLP PLFNFSAGEL SIQPSLNAIL IPPHLRS	
401	

Fig. 7